

SYNTHESIS AND CHIROPTICAL PROPERTIES OF SOME GLYCEROLIPIDS

Peter Michelsen



Lund 1981



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Abstract Optically active derivatives of 1-thioglycerol, starting from 1,2-0-isopropylidene-sn-glycerol have been synthesized. The chiroptical properties were studied by Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD). It was demonstrated that these compounds have much stronger optical activity than the corresponding glycerol derivatives that do not contain sulphur. They were also used for the study of the metabolism of enantiomeric glycerides. Trioleoyl-3-thio-sn-glycerol and 1,2-0-dioleoyl-3-S-tetra-decyl-3-thio-sn-glycerol-S-oxide were used as triacyl glycerol analogues and were analyzed for their stereoconfiguration by ORD/CD. The synthesis and chiroptical properties of glyceryl ethers are described. A method for the synthesis of 1,3-0-dialkyl-sn-glycerol was developed, which involves selective alkylation of 3-0-alkyl-sn-glycerol to the desired compound. The chiroptical properties of some phospholipids have been studied by ORD and CD. The mass spectra of some 1,2-0-dialkyl- and 1,3-0-dialkylglycerols have been recorded. They can be distinguished from each other by their different fragmentation patterns. The mass spectra of all positional isomers of dl-hydroxyoctadecanoyl pyrrolidines are recorded. All spectra of the different isomers can be distinguished from each other. 			
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Signature *Peter Michelsen* Date September 7, 1981

CONTENTS

	page
1. INTRODUCTION	
1.1 <i>General aspects</i>	5
1.2 <i>Nomenclature</i>	5
1.3 <i>Optical activity of glycerol derivatives</i>	6
1.4 <i>Mass spectrometry</i>	7
1.5 <i>Synthesis of optically active starting material</i>	7
2. SYNTHESIS OF TRIACYLGLYCEROLS	9
3. SYNTHESIS AND CHIROPTICAL PROPERTIES OF DERIVATIVES OF 1-THIOGLYCEROL	11
3.1 <i>Glycerides with a thio ester bond</i>	11
3.2 <i>Glycerides containing sulphide, sulphoxide or sulphone groups</i>	16
4. CHIROPTICAL PROPERTIES OF SOME PHOSPHOLIPIDS	20
5. SYNTHESIS AND CHIROPTICAL PROPERTIES OF GLYCERYL ETHERS	
5.1 <i>Introduction</i>	28
5.2 <i>Chiroptical properties of 1,2-diacyl-3-O-alkyl-sn-glycerols</i>	28
5.3 <i>Synthesis and chiroptical properties of acyl-O-dialkyl-sn-glycerols</i>	30
6. MASS SPECTROMETRIC ANALYSIS	35
6.1 <i>Mass spectra of some glyceryl ethers</i>	35
6.2 <i>Mass spectrometric studies of isomeric hydroxy fatty acid pyrrolidides</i>	36
7. METHODS FOR STUDIES OF THE METABOLISM OF ENANTIOMERIC GLYCERIDES	42
7.1 <i>Introduction</i>	42
7.2 <i>Intestinal absorption of lipids</i>	43
7.3 <i>Triacylglycerol analogue with thio ester bond for the study of stereospecificity in lipid absorption</i>	43
7.4 <i>1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol- S-oxide as a triacylglycerol analogue for the study of stereospecificity in lipid absorption</i>	45
7.5 <i>Stereospecificity of phospholipases</i>	46
8. ACKNOWLEDGEMENTS	48
9. REFERENCES	49

This thesis is a summary of the following papers.
Throughout the thesis they are referred to by their Roman numerals.

- I. P. Michelsen and B. Herslöf
Synthesis of Some Triacylglycerols in Palm Oil.
Manuscript
- II. S. Gronowitz, B. Herslöf, P. Michelsen and B. Åkesson
Syntheses and Chiroptical Properties of Some Derivatives
of 1-Thioglycerol.
Chem. Phys. Lipids 22 (1978) 307
- III. P. Michelsen, B. Herslöf and B. Åkesson
Chiroptical Properties of S-Alkyl Derivatives of
1-Thioglycerol.
Chem. Phys. Lipids, in press
- IV. P. Michelsen, S. Gronowitz, B. Åkesson and B. Herslöf
Chiroptical Properties of Some Phospholipids.
Manuscript
- V. P. Michelsen and B. Herslöf
Synthesis and Chiroptical Properties of Glyceryl Ethers.
Manuscript
- VI. P. Michelsen and B.Å. Andersson
Mass Spectra of Some Glyceryl Ethers.
Manuscript
- VII. P. Michelsen and B.Å. Andersson
Pyrrolidide Derivatives for Mass Spectrometric Studies
of Isomeric Hydroxy Fatty Acids.
Manuscript
- VIII. B. Åkesson, S. Gronowitz and P. Michelsen
Digestion and Absorption of Trioleoyl-Thioglycerol
in the Rat.
Chem. Phys. Lipids 23 (1979) 93
- IX. B. Åkesson and P. Michelsen
Digestion and Absorption of A Sulphoxide Analogue of
Triacylglycerol in the Rat.
Manuscript

1. INTRODUCTION

1.1 *General aspects*

Lipids can be subdivided into two broad classes: "simple" lipids such as triacylglycerol, diacylalkylglycerol and other fatty acid esters, and "complex" ones such as phospholipids, dependent of the number of moieties liberated by hydrolysis.¹

In many biochemical reactions, enzymes act selectively on glycerolipids, and in many cases the stereo structure of glycerolipids is important. Many of the naturally occurring glycerolipids are optically active, due to the existence of one or several asymmetric carbon atoms. For the study of problems in lipid analysis, biochemistry and nutrition, it is of great importance to have well defined synthetic model substances and to have several methods for the establishment of the structure and the configuration of these compounds. This can be done by NMR, MS and ORD/CD, as well as with other techniques, such as the use of different lipases and chemical degradations. In this thesis, the main purpose was to use MS and ORD/CD.

1.2 *Nomenclature*

Several different methods have been used to specify the absolute configuration of derivatives of glycerol. That by Hirschman² introduced Stereospecific Numbering System (the sn-system), has been used for the most part in this thesis.

In this system, glycerol is numbered from the "top" to "bottom", if the secondary hydroxyl group is shown to the left of carbon 2 in the Fischer projection (Fig. 1). The use of the stereospecific numbering is indicated by the prefix sn- before the root name glycerol.

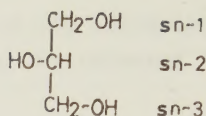


Fig. 1. Fischer projection of glycerol and the sn-numbering of the molecule

1.3 *Optical activity of glycerol derivatives*

Glycerol by itself is not optically active, but if groups attached to the terminal hydroxyls of glycerol differ from each other structurally (such as difference of chain length, chromophores, asymmetric centra etc.), it can be resolved into two enantiomeric forms i.e. they are optically active.

Optical activity is associated with electronic transitions of a chromophore occurring in a chiral environment. Since the optical activity varies as a function of wavelength, it can be measured by spectroscopic techniques, such as Optical Rotatory Dispersion (ORD) or Circular Dichroism (CD).³ These techniques have been widely used in organic chemistry to determine the absolute or relative configurations and conformations, since the 1950:s when the first instruments became available.

Relatively few lipids have been studied with these techniques. At this institute, several enantiomeric acyl-glycerols and their derivatives have been studied by Herslöf with ORD and CD.⁴ The results indicated that it should be possible to use these techniques for the stereochemical analysis of a wider range of lipids.

1.4 Mass spectrometry

Among the methods for the determination of the structure of lipids, mass spectrometry (MS) is a very useful tool. This was made possible by the pioneering work by Ryhage and Stenhagen in the 1960:s.⁵

In this thesis MS has been used for two major reasons:

- A. For the confirmation of the structures of the synthesized compounds. The mass spectra obtained were compared with those known from the literature or with known fragmentation patterns. The analysis of the spectra was not discussed when the information was obvious.
- B. For the determination of positional isomers and the elucidation of the fragmentation patterns of some glyceryl ethers (Paper VI) and of some isomeric hydroxy fatty acid pyrrolidides (Paper VII).

1.5 Synthesis of optically active starting material

In order to obtain optically active derivatives of glycerol for the study of biochemical reactions and for our continuing investigations of the chiroptical properties of lipid derivatives, the fundamental stereospecific synthesis introduced by Fischer and Baer in 1939⁶ was used (Fig. 2).

D-Mannitol, which occurs naturally and is commercially available, was converted by reaction with acetone in the presence of zinc chloride to 1,2:5,6-diisopropylidene-D-mannitol. By oxidative cleavage with sodium metaperiodate, the protected glyceraldehyde was obtained, which through reduction with sodium borohydride gave 1,2-isopropylidene-sn-glycerol. This compound was the main precursor for the various optically active derivatives of glycerol studied in this thesis.

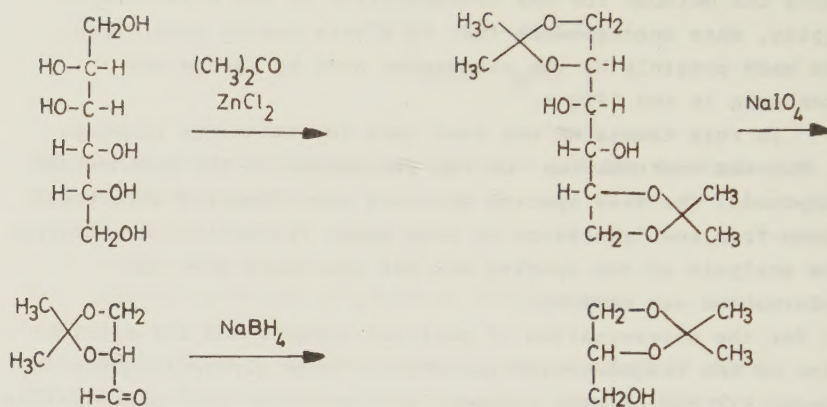


Fig. 2. Synthesis of 1,2-O-isopropylidene-sn-glycerol according to Fischer and Baer⁶

2. SYNTHESIS OF TRIACYLGLYCEROLS

From the technical point of view, it is very important to obtain information about the quality of plant oils and to improve their quality before industrial use.

Schlenk et al.^{7,8} have shown that enantiomeric triacylglycerols can be distinguished from the corresponding racemic compounds by the X-ray diffraction pattern technique. This technique has been used extensively in the investigation of acylglycerols structure because of the information one can obtain from the polymorphic nature of their crystals. Thereby the qualities of different products of plant oils can be improved. Together with AB Karlshamns Oljefabriker, Karlshamn, we intend to examine palm oil with this technique (Michelsen et al., to be published). For this purpose we prepared ten triacylglycerols, which are major components of palm oil, by some of the many methods for stereospecific synthesis of triacylglycerol. This work is described in Paper I.

Examples of the synthetic routes in this work are given in Fig. 3, where the syntheses of the two enantiomeric diacid-triacylglycerols are shown. Route b in Fig. 3 is especially useful for syntheses of triacylglycerols with three different fatty acids, but other methods described in Paper I can also be used.

For review of the stereospecific syntheses of enantiomeric acylglycerols, see Ref. 9 and references therein.

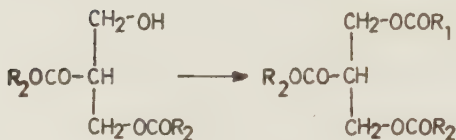
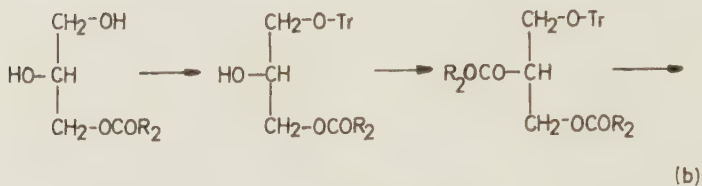
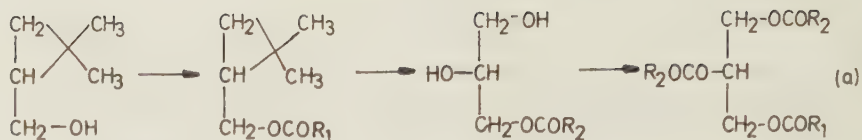


Fig. 3. The synthesis of enantiomeric triacylglycerols

3. SYNTHESIS AND CHIROPTICAL PROPERTIES OF DERIVATIVES OF 1-THIOGLYCEROL

Since triacylglycerols and diacylalkylglycerols have too small rotations in CD (see Table 1) to be useful for the analysis of small amounts of biological samples, there was a need for triacylglycerol analogues with high optical activity. For our continuing investigations of the chiroptical properties of lipid derivatives, a study of derivatives of 1-thioglycerol was of interest since a more measurable effect could be expected. Several such derivatives were therefore synthesized, which is summarized in Table 2. The results of this work has been published in Papers II and III. It is also of interest that acylglycerols containing the thiol ester bond have been suggested as suitable substrates for the assay of lipase activity.¹⁰ It has also been reported by Ferrel et al.¹¹ that S-alkylglycerol lipids occur in nature, especially in bovine heart.

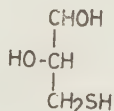


Fig. 4. 1-Thioglycerol (3-thio-sn-glycerol)

3.1 *Glycerides with a thio ester bond*

In Paper II, 1,2-O-isopropylidene-sn-glycerol was used as starting material for the synthesis of 1,2-O-isopropylidene-3-thio-sn-glycerol, which was the basic precursor for the synthesis of optically active derivatives of 1-thioglycerol (Fig. 5).

	$[\Theta]_{\max}$	Amount
$ \begin{array}{c} \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{C}_{17}\text{H}_{35} \\ \\ \text{CH}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{C}_{17}\text{H}_{35} \\ \\ \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}\text{CH}_3 \end{array} $	- 64	~ 100-200mg
$ \begin{array}{c} \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{C}_{17}\text{H}_{35} \\ \\ \text{CH}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{C}_{17}\text{H}_{35} \\ \\ \text{CH}_2-\text{S}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3 \end{array} $	- 3600	~ < 5mg
$ \begin{array}{c} \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}_2-\text{O}-\text{C}_6\text{H}_{13} \end{array} $	- 490	~ 100mg
$ \begin{array}{c} \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}_2-\text{S}-\text{C}_{14}\text{H}_{29} \end{array} $	- 1380	~ < 10mg
$ \begin{array}{c} \text{CH}_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R} \\ \\ \text{CH}_2-\text{S}-\text{C}_{14}\text{H}_{29} \\ \downarrow \\ \text{O} \end{array} $ (R,R)	- 17260	~ < 1,5 mg

Table 1. The maximum optical activity by CD and the amount of samples required for recording spectra of some optically active glycerol derivatives

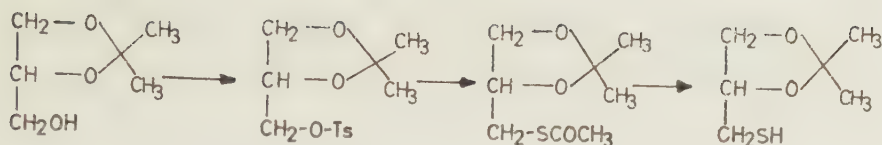


Fig. 5. Synthetic route to 1,2-O-isopropylidene-3-thio-sn-glycerol

From this compound triacylglycerol analogues, 1,2-O-diacyl-3-S-acyl-3-thio-sn-glycerols were synthesized, see Table 2, in the same manner as the triacylglycerols that do not contain sulphur.

In Paper II, we have also determined the optical purity of 1,2-O-isopropylidene-3-S-acetyl-3-thio-sn-glycerol by ¹H NMR and a chiral shift reagent. Analysis of the ¹H NMR spectrum by computer-simulated Lorentzian curves showed that the compound had an optical purity higher than 99 %. Thus, this observation confirms that the previous steps produce optically pure products, which is important, since there are different opinions about the optical purity of 1,2-O-isopropylidene-sn-glycerol¹² and consequently of compounds prepared from it.

In the interpretation of the CD curve of triacylglycerol (Fig. 6), only the n→π* transition at 210 nm of the ester chromophore appeared (the lonely pair electrons of the C=O oxygen),⁴ while the corresponding 3-thio-sn-glycerol derivative shows a red shift (Fig. 6). The absorption at 235 nm has been assigned to a π→π* transition, while the band at 270 nm, because of its low intensity and its solvents dependence, has been assigned to the n→π* transition of the thio ester chromophore (S-C=O).¹³ Figure 6 also demonstrates that the thio analogue of the triacylglycerol has much stronger optical activity than the corresponding triacylglycerol. Consequently, the amount of sample needed for recording a spectrum is very small, which is important for the analysis of small amounts of biological samples.

Table 2. Synthesized optically active derivatives of 1-thioglycerol

Derivative	$[\alpha]_{\text{max}}$	Solvent
1,2-O-Isopropylidene-3-thio-sn-glycerol	220 (220)	ethanol
1,2-O-Isopropylidene-3-S-acetyl-3-thio-sn-glycerol	2600 (200)	hexane
1,2-O-Isopropylidene-3-S-oleoyl-3-thio-sn-glycerol	2600 (220)	"
Bis-1,2-O-isopropylidene-3-thio-sn-glycerol	3700 (190)	"
3-Thio-sn-glycerol	-120 (225)	ethanol
Bis-3-thio-sn-glycerol	-6300 (205)	"
3-S-Acetyl-3-thio-sn-glycerol	-2800 (230)	"
3-S-Oleoyl-3-thio-sn-glycerol	-2800 (230)	"
1,2-O-Distearoyl-3-S-acetyl-3-thio-sn-glycerol	-3600 (230)	hexane
1,2-O-Diacetyl-3-S-acetyl-3-thio-sn-glycerol	-4300 (225)	"
1,2-O-Dioleoyl-3-S-oleoyl-3-thio-sn-glycerol	-3300 (225)	"
1,2-O-Isopropylidene-3-S-tetradecyl-3-thio-sn-glycerol	-5000 (200)	"
3-S-Tetradecyl-3-thio-sn-glycerol	1600 (200)	ethanol
1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol	-1380 (225)	hexane
1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol-S-oxide	-17260 (210)	"
1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol-S-oxide	-9650 (215)	"
1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol-S-dioxide	-240 (205)	"

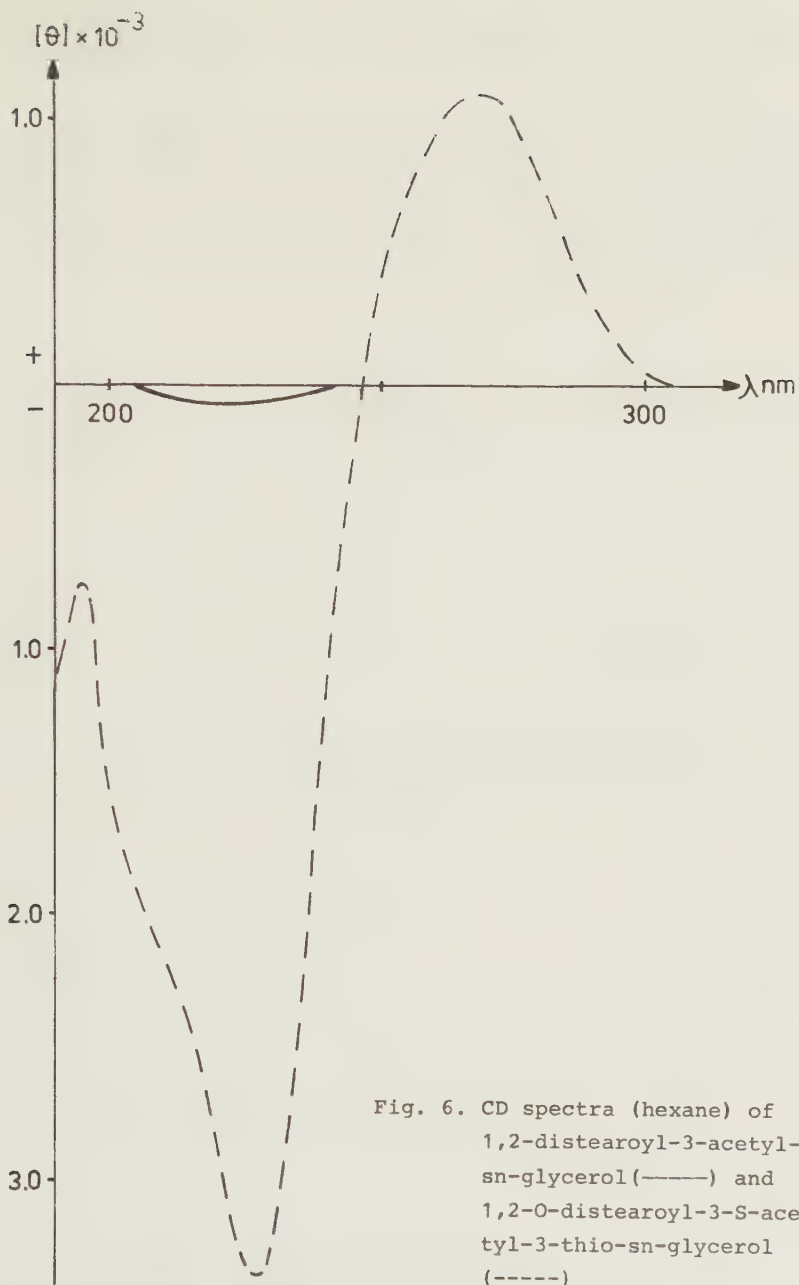
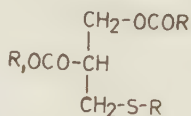


Fig. 6. CD spectra (hexane) of
 1,2-distearoyl-3-acetyl-
 sn-glycerol(——) and
 1,2-O-distearoyl-3-S-ace-
 tyl-3-thio-sn-glycerol
 (-----)

3.2 Glycerides containing sulphide, sulphoxide or sulphone groups

In Paper III, the synthesis and chiroptical properties of glycerylthio ether derivatives are described. Several 1,2-O-diacyl-3-S-alkyl-3-thio-sn-glycerols were prepared as analogues to 1,2-diacyl-3-O-alkyl-sn-glycerols (Fig. 7). Some of the compounds synthesized are summarized in Table 2.



The structure of 1,2-O-diacyl-3-S-alkyl-3-thio-sn-glycerol

It was found that the glycerylthio ethers had higher optical activity than the corresponding O-alkyl derivatives (see Table 1), but the difference is not as pronounced as triacylglycerols compared to triacylthioglycerols.

The CD spectrum of 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol (Fig. 8) shows a negative maximum at 225 nm, which can be assigned to the $n \rightarrow \pi^*$ transition of the ester chromophore. The CD spectra of the 1,2-diacyl-3-O-alkyl-sn-glycerols (see page 29) and the 1,2-diacyl-sn-glycerol also showed negative maxima at 215 nm.

From 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol, the sulphoxide and the sulphone were obtained by oxidation with hydrogen peroxide (Fig. 9).

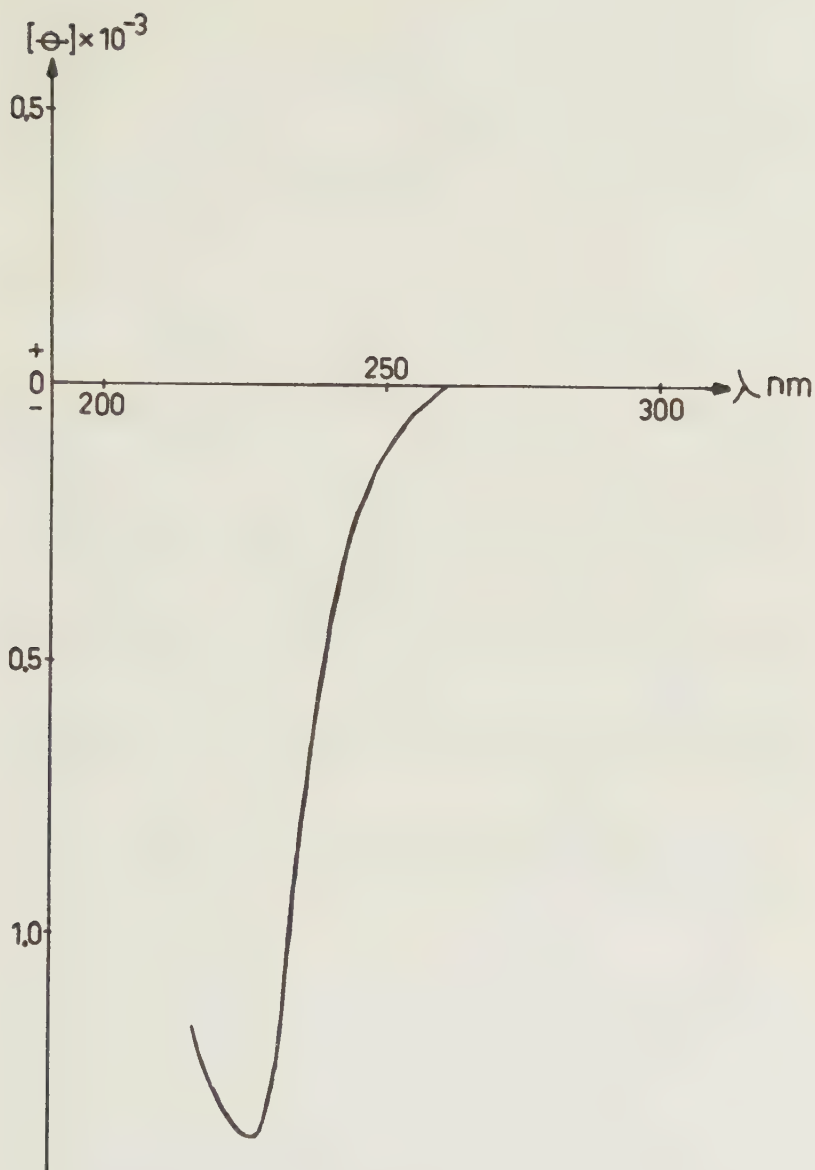


Fig. 8. CD spectra (hexane) of 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol

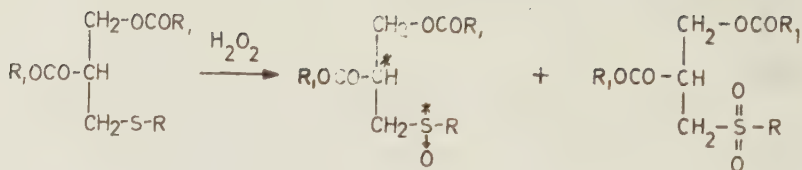


Fig. 9. Conversion of the sulphide to the corresponding sulphoxide (* chiral centres) and sulphone

Since unsymmetrically substituted sulphoxides are chiral, this compound could be resolved into its two diastereomers, which have the absolute configuration R,R and R,S, respectively. By comparison of their ORD curves (Fig. 10) with those for simple alkyl-methyl sulphoxides,¹⁴ we could tentatively establish the absolute configuration of these two compounds.

For simple alkyl-methyl sulphoxides it has been demonstrated¹⁴ that a negative Cotton effect centred near 200 nm indicates the R-configuration. Applying this to diastereomer A in Fig. 10 would therefore imply that the sulphoxide group has the R-configuration and the compound has the R,R-configuration, and consequently diastereomer B has the S,S-configuration.

These compounds have also been used for studies of the stereochemistry of fat digestion (Paper IX, page 45).

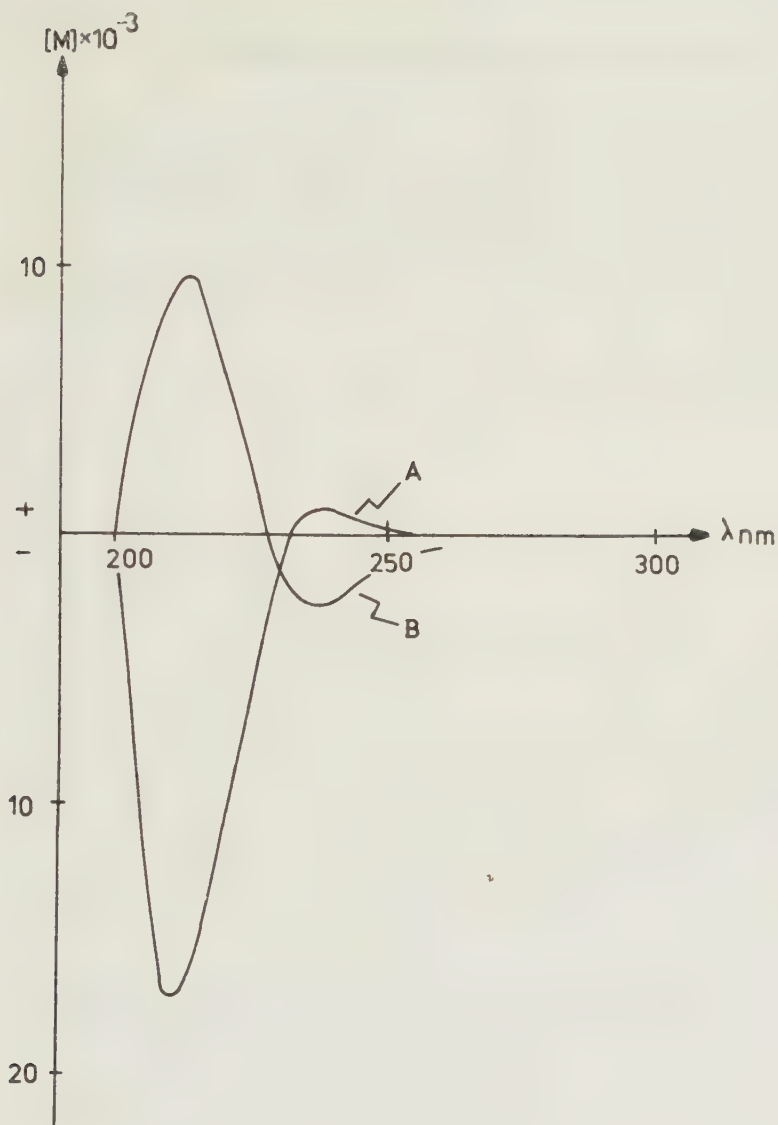


Fig. 10. ORD spectra (hexane) of the two diastereomers of 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol-S-oxide, where A and B have the absolute configuration R,R and R,S, respectively

4. CHIROPTICAL PROPERTIES OF SOME PHOSPHOLIPIDS

Phospholipids are found in all plant and animal tissues and in microorganisms, especially in various cell membranes, where they are involved in many biochemical reactions.

In Paper IV, a number of phospholipids with the general structures shown in Fig. 11 have been examined by ORD and CD.

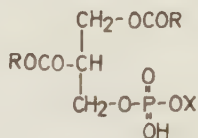


Fig. 11. General structure of phospholipids
(X = H or choline, ethanolamine etc.)

Present methods available to establish the stereoconfiguration of phospholipids involve the use of different lipases and chemical degradations. These methods are often tedious, demand large amounts of sample and are destructive.

Our intention was to systematically investigate the chiroptical properties of some of these compounds to establish whether ORD and CD could be suitable analytical techniques in studies of their stereochemistry.

Batrakow et al.¹⁵ have studied the CD spectra of some synthetic and naturally occurring phospholipids. From their data, complemented by NMR studies, they postulated the rule that if the phosphate group is situated at the sn-3 position of glycerol, the CD sign at 215-224 nm should be positive. We have reinvestigated some of the compounds and our results differed from the previous data, since we obtained a negative sign of the CD effects. The results are summarized in Table 3.

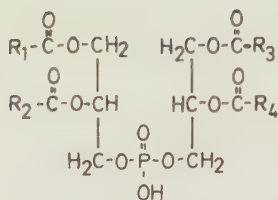
Table 3. The CD sign of some of the investigated phospholipids

Compound	CD sign	λ_{max}	Solvent
Diacyl-sn-glycero-3-phosphate	- ^{a,b}	225	dioxane HF-2-P
Diacyl-sn-glycero-3-phosphocholine (lecithin)	+ + ^b - - ^a	210 218 210	HF-2-P methanol ethanol heptane
Diacyl-sn-glycero-3-phospho-1'-sn-glycerol (phosphatidyl glycerol)	- ^a	220	heptane
Tetraacyl-sn-glycero-3-phosphoryl-3'-sn-glycerol (bis(diacylglycerol)phosphate)	-	210	hexane
1-[1,2-Diacyl-sn-glycero-(3)-phospho]-3-[1,2-diacyl-sn-glycero-(3)-phospho]-sn-glycerol (cardiolipin)	- ^a - -	230 230 230	heptane ethanol TFE

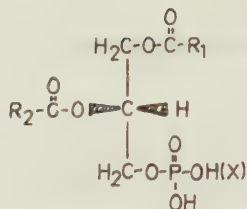
^aFor these samples positive CD signs were reported by Batrakov et al.¹⁵

^bNo maxima were obtained.

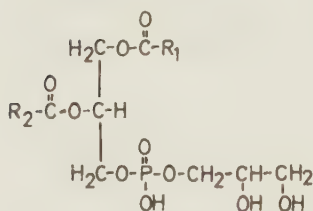
Abbreviations: HF-2-P, 1,1,1,3,3,3-hexafluoro-2-propanol
TFE, 1,1,1-trifluoroethanol



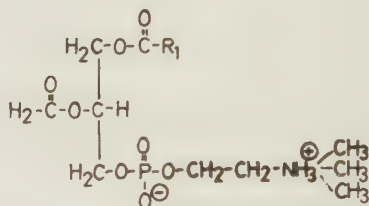
tetraacyl-sn-glycero-3-phosphoryl-3'-sn-glycerol
(bisphosphatidic acid)



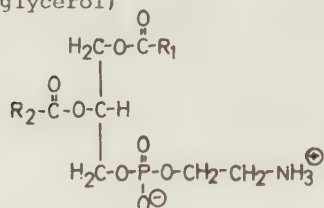
diacyl-sn-glycero-3-phosphate



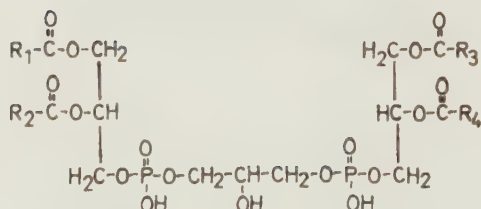
diacyl-sn-glycero-3-phospho-1'-sn-glycerol (phosphatidyl glycerol)



diacyl-sn-glycero-3-phosphocholine (lecithin)



diacyl-sn-glycero-3-phosphoethanolamine



1-(1,2-diacyl-sn-glycerol-(3)-phospho)-3-(1,2-diacyl-sn-glycerol-(3)-phospho)-sn-glycerol (cardiolipin)

Fig. 12. Compounds investigated

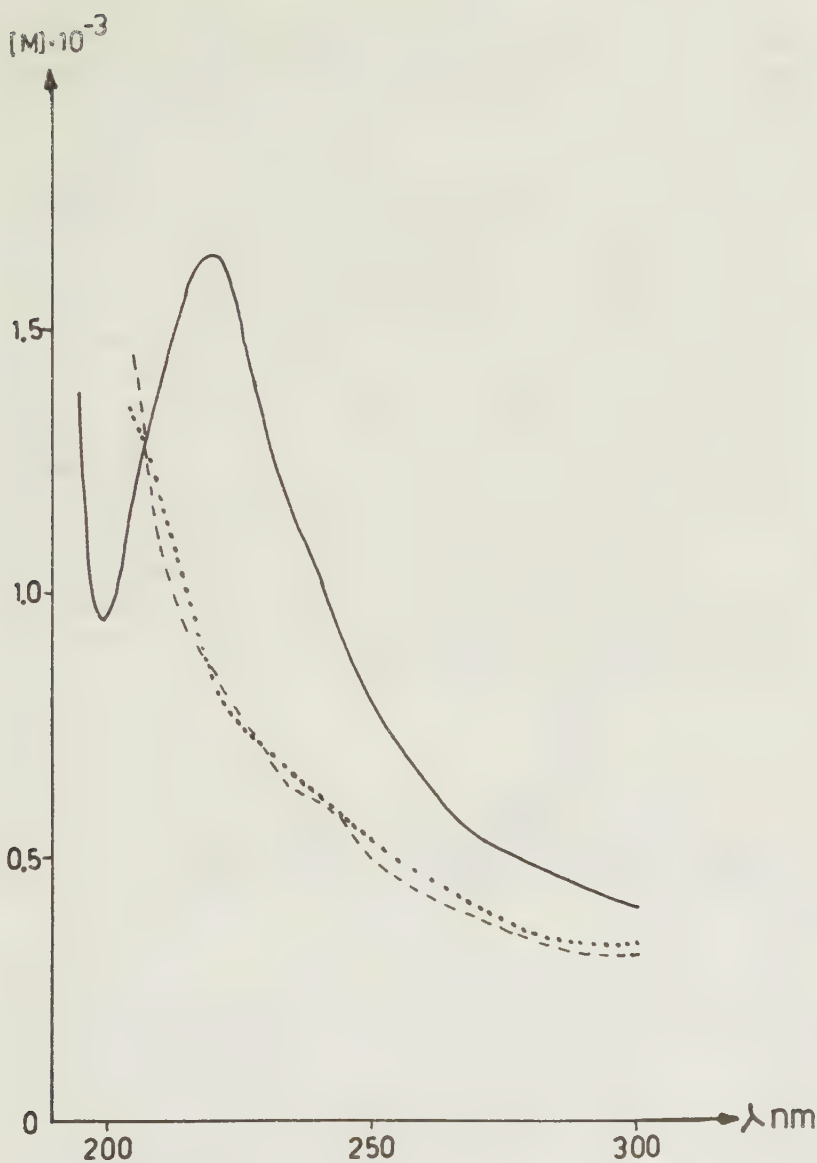


Fig. 13. ORD spectra of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (—, hexafluoroacetone), (----, ethanol), (....., methanol)

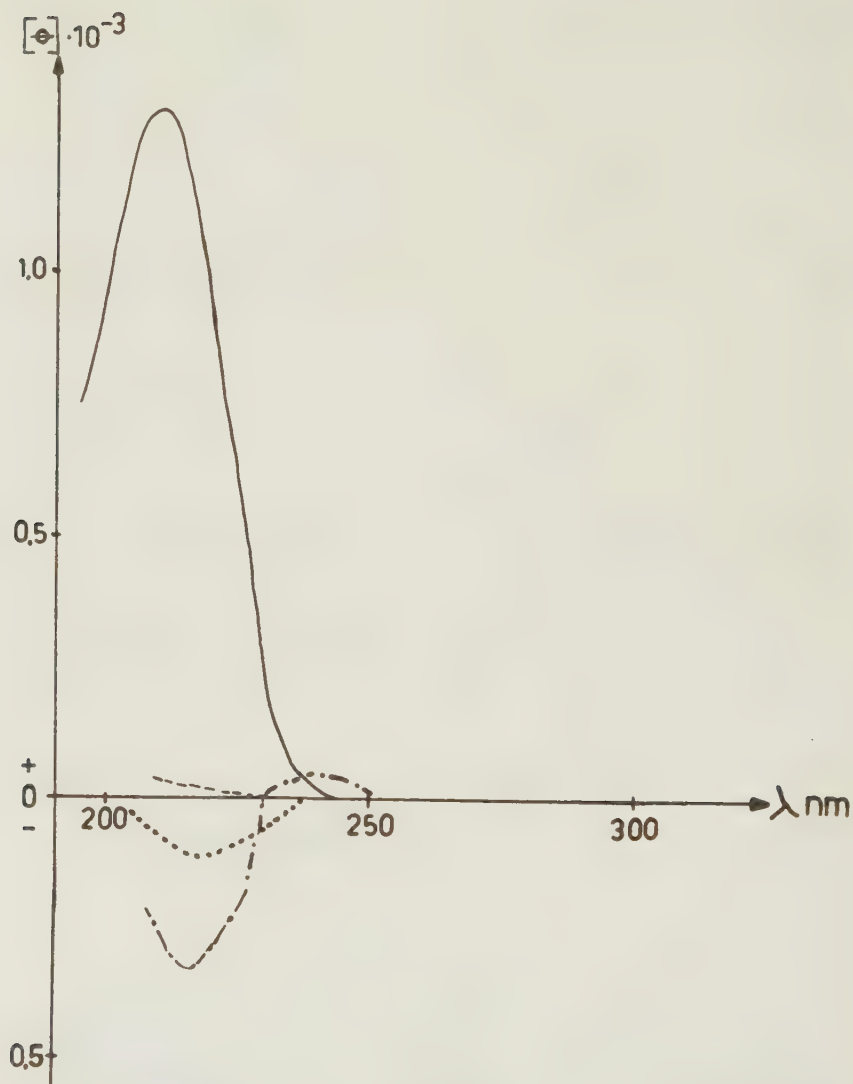


Fig. 14. CD spectra of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (—, hexafluoroacetone), (----, methanol), (····, ethanol) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (-·-·-, heptane)

Our data show that if the phosphate group is situated at the sn-3 position, the ORD rotations are positive, independent of the solvent used, for all of the structures investigated (Fig. 12).

However, since ORD does not always show clearly recognizable Cotton effects depending on the solvent used (Fig. 13), the most useful spectroscopic method is CD, since it most often shows clearly recognizable maxima or minima (Fig. 14).

The CD curves of the compounds investigated (their signs are summarized in Table 3) show a negative CD effect, if either ethanol, heptane, hexane or dioxane is used as solvent. This CD effect is due to the $n \rightarrow \pi^*$ transition of the ester chromophore. If a fluorinated solvent is used, 1,2-diacyl-sn-glycerophospho-choline and -ethanolamines show a positive CD effect, but the other compounds, such as cardiolipin and phosphatidylglycerol, still have a negative CD effect. Thus it might be possible to distinguish these types of compounds from each other by choosing a fluorinated solvent.

There are some doubts about the configuration of the naturally occurring tetraacyl-sn-3-phosphoryl-3'-sn-glycerol¹⁶ (Fig. 15).

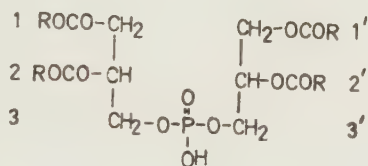


Fig. 15. The structure of tetraacyl-sn-glycero-3-phosphoryl-3'-sn-glycerol

It might have the 3-sn-1'-sn-glycerol configuration as for phosphatidylglycerol (diacyl-sn-glycero-3-phospho-1'-sn-glycerol, see Fig.12), but there are difficulties with the present methods to determine its absolute configuration.

However, we have used the CD technique to determine the configuration of synthetic samples, kindly supplied to us

by Dr. H. Schmid, Hormel Institute, USA.

CD spectra were taken of pure samples of tetrapalmitoyl-sn-glycero-3-phosphoryl-3'-sn-glycerol and of a mixture of the 3-sn-3'-sn-glycerol and 3-sn-1'-sn-glycerol isomers (Fig. 16). It was found that the rotation for the pure 3-sn-3'-sn-glycerol isomer was twice that for the sample containing the mixture of isomers. This was expected, since the latter sample should contain an equal mixture of each isomer. The results demonstrate that CD might be a very useful tool to solve this kind of structure problem, at least in pure compounds.

In preliminary experiments the CD technique has been used to elucidate the stereospecificity of phospholipase D, as discussed in Section 7.5.

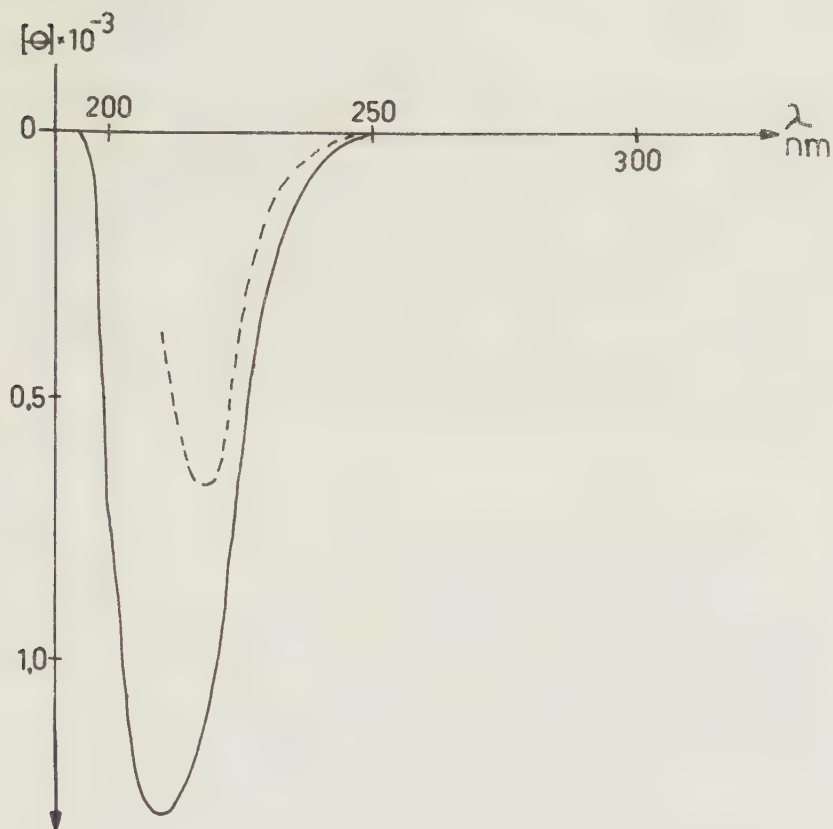


Fig. 16. CD spectra (hexane) of tetrapalmitoyl-*sn*-glycero-3-phosphoryl-3'-*sn*-glycerol (—) and a mixture of 3-*sn*-3'-*sn*-glycerol/3-*sn*-1'-*sn*-glycerol isomers (-----)

5. SYNTHESIS AND CHIROPTICAL PROPERTIES OF GLYCERYL ETHERS

5.1 Introduction

Cymerman-Craig¹⁷ was the first to use ORD to relate the absolute configuration of naturally occurring plasmalogens to each other. By comparison of the optical rotations of synthetic 1,2-diacyl-3-O-alkyl-sn-glycerols with those of naturally isolated compounds, Bauman et al.¹⁸ could later on establish the absolute configurations of these compounds.

In order to perform a more detailed study of glyceryl ethers with a more sensitive ORD and CD equipment, a number of different O-alkylglyceryl ethers has been synthesized. The synthesis and chiroptical properties of these compounds are described in Paper V.

5.2 Chiroptical properties of 1,2-diacyl-3-O-alkyl-sn-glycerol

When the chain length of the fatty acids of 1,2-diacyl-3-O-alkyl-sn-glycerol was varied, the optical activity remained almost unchanged (1,2-diacetyl- and 1,2-dilauroyl-3-O-hexyl-sn-glycerol, Fig. 17). As described in Paper V, it has also been demonstrated that 1,2-dipalmitoyl- and 1,2-dimyristoyl-3-O-benzyl-sn-glycerol show the same optical activity.

If the alkyl chain was increased from hexyl to hexadecyl (1,2-dilauroyl-3-O-hexyl-sn-glycerol and 1,2-dimyristoyl-3-O-hexadecyl-sn-glycerol) only a small effect on the optical activity was observed (Fig. 17). When the alkyl was methyl, the activity decreased, and when the alkyl group was replaced by hydrogen, i.e. 1,2-dimyristoyl-sn-glycerol the optical activity was lower (Fig. 17). This is probably due to the fact that the number of possible conformations is larger, when the alkyl chain is very short at the sn-3 position. Only when the alkyl chain contains a strong chromophore such as in 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol, does stronger optical activity arise (Fig. 17).

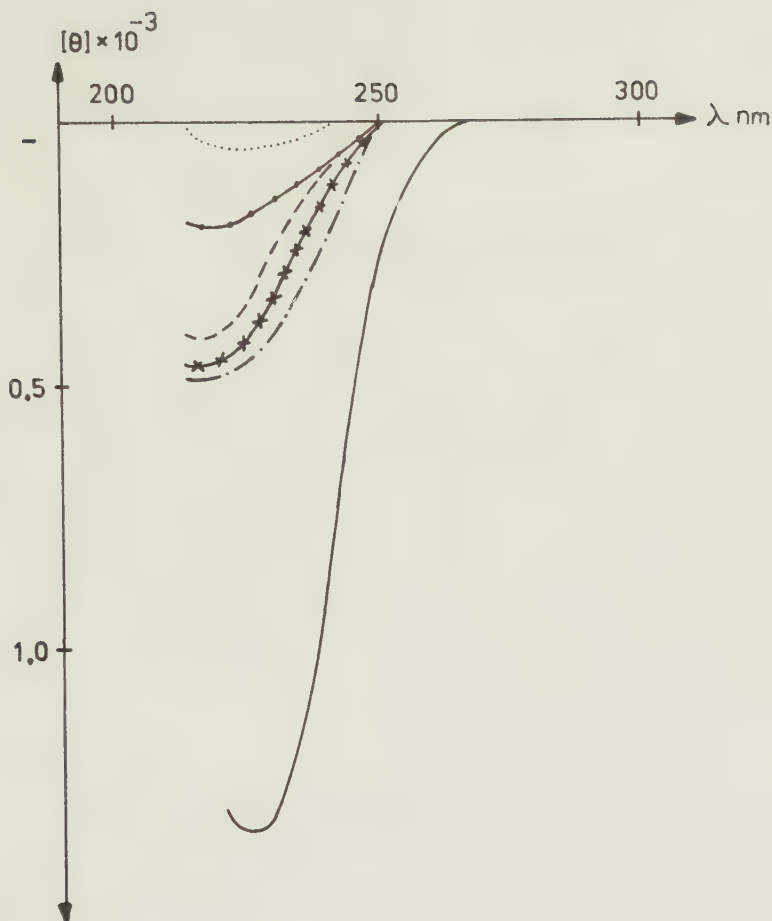


Fig. 17. CD spectra (ethanol) of 1,2-dimyristoyl-sn-glycerol (.....), 1,2-dimyristoyl-3-O-methyl-sn-glycerol (•••••), 1,2-diacetyl-3-O-hexyl-sn-glycerol (-----), 1,2-dilauroyl-3-O-hexyl-sn-glycerol (*-*-*), 1,2-dimyristoyl-3-O-hexadecyl-sn-glycerol (-·-·-) and 1,2-O-dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol (—) (hexane)

The CD curves of all 1,2-diacyl-3-O-alkyl-sn-glycerols investigated have the same shape (Fig. 17). They exhibit negative Cotton effects around 215 nm which are assigned to the $n \rightarrow \pi^*$ transitions of the ester chromophores. This is also the observation for mono- and diacyl-sn-glycerols described elsewhere.⁴

5.3 *Synthesis and chiroptical properties of acyl-O-dialkyl-sn-glycerols*

During 1975 Batrakov et al.¹⁵ published a paper on the CD spectra of some natural and synthetic phosphatides. The authors noticed a Cotton effect at 215-226 nm, which they assigned to the $n \rightarrow \pi^*$ transition of the ester chromophore directly bound to the asymmetric carbon atom of glycerol, i.e. the sn-2 position. They applied the carbonyl sector rule¹⁹ to the compounds investigated, assuming them to have a spherical structure and could thus calculate the expected sign of the Cotton effect. As discussed in Section 4, we have obtained the opposite sign for some of these phospholipids and therefore it is very doubtful if the carbonyl sector rule can be applied on glycerol derivatives.

In order to investigate how the CD sign is influenced by the carbonyl attached at different positions, both enantiomeric forms of 2-acyl-1,3-O-dialkyl-sn-glycerol and the 3-acyl-1,2-dialkyl-sn-glycerol were synthesized.

The method²⁰ we first used for the synthesis of 1,3-O-dialkyl-sn-glycerol is outlined in Fig. 18. This method appeared to be tedious and the overall yield was very low, less than 5 %.

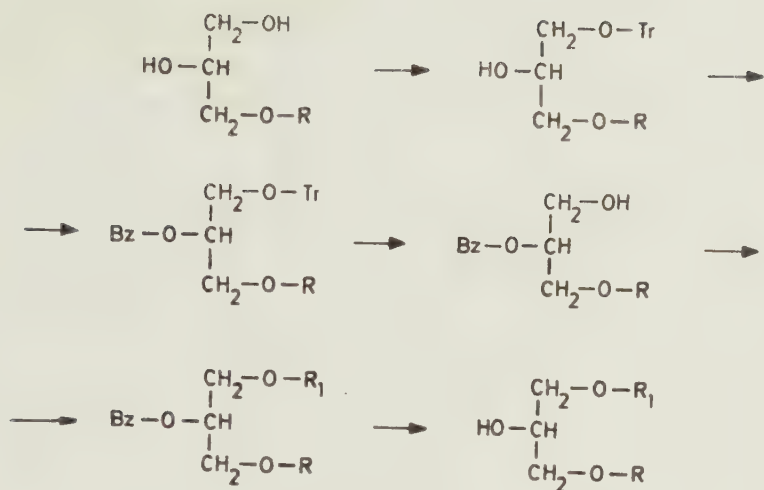


Fig. 18.

Instead we developed a method which involves selective alkylation of a 3-O-alkyl-sn-glycerol to the desired compound according to Fig. 19 and the overall yields were 40 %.

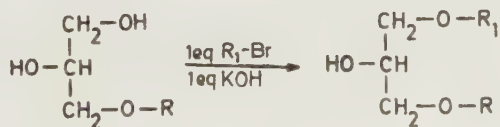


Fig. 19.

The chiroptical properties of all compounds investigated (see Table 4 and 5), shows that there is a correlation between the sign of the observed CD-effect at 213-225 nm and the position of the fatty acid residue in the glycerol derivative. These Cotton effects are due to the $n-\pi^*$ transition of the ester chromophore.

From the compounds reported here and in previous work, it is obvious that the fatty acid in the sn-1 position gives a negative measured effect and the reverse result is obtained by the fatty acid at the sn-3 position. This explains the very small effects observed in triacyl-sn-glycerols since the effects from the sn-1 and sn-3 "neutralize" each other⁴. However, in monoacyl- and diacyl-sn-glycerols or in mixed alkyl-acylglycerols the separate effects from each sn-position can be studied. The 2-acyl-1,3-0-dialkyl-sn-glycerols investigated in this paper make the role of the sn-2 position clearer. Depending on the difference in chain length between the terminal 0-alkyl groups, the sign of the Cotton effects from the sn-2 fatty acid residue can either be negative or positive (cf. Table 4).

It is clear that the observed CD effect of 1,2-diacyl-sn-glycerol derivatives is composed of two effects, one from each ester chromophore. These can cooperate to a large effect or counteract each other to a small effect. This is probably the explanation of the great difference in magnitudes of the CD effects observed in different solvents, ie. diacylglycerols⁴.

The data from this and previous papers show that the measured CD sign is determined by the structural position of the terminal fatty acid in the glycerol derivative. By this it is clear that Batrakow et al.¹⁵, probably only have determined the sn-2 fatty acid influence and thereby obtained misleading information.

Table 4. Physical data and rational values for:

Compound	Yield %	mp°C	[M]	λ	$[\theta]_{\max}$	$\lambda_{\max}$	Solvent
sn-MM-3-O-methyl	47	34-35	+ 925	205	- 197	215	ethanol
sn-AcAc-3-O-hexyl	47		+1097	210	- 422	215	"
sn-LaLa-3-O-hexyl	79		+1904	200	- 490	215	"
sn-MM-3-O-hexadecyl	49	49.0-49.5	+1747	200	- 496	215	"
sn-MM-3-O-benzyl	65	33-34	+1551	220	- 277 +2250	225 213	hexane
sn-PP-3-O-benzyl	77	43-44	+2292 +1042	215 210	- 278 +2100	225 213	"
sn-AcAc-3-O-methylthiomethyl	87		+ 476	250	- 194	220	ethanol
sn-MM-3-O-methylthiomethyl	83	27-28	+ 592	250	- 160 ^a	225	"
sn-La-1-O-hexyl-3-O-hexadecyl	95				- 76	215	"
sn-La-1-O-hexadecyl-3-O-hexyl	85				+ 76	215	"
sn-La-1,2-O-dihexadecyl	83				+ 172	215	hexane

Abbreviations: Ac acetic, La lauric, M myristic, P palmitic.

^ano maximum was obtained.

Table 5. CD values and sign for:

Compound	$[\theta]_{\max}$	$\lambda_{\max}$	Solvent
1-Myristoyl-sn-glycerol ⁴	- 160 ^a	213	ethanol
1,2-Dimyrystoyl-sn-glycerol	- 159	218	hexane
	- 60	218	ethanol
1,2-Dimyrystoyl-3-O-hexyl-sn-glycerol	- 495	215	"
1,2-Dimyrystoyl-3-O-benzyl-sn-glycerol	- 280	225 ^b	hexane
1-Lauroyl-2,3-O-dihexadecyl-sn-glycerol	- 170 ^a	215	"
1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol	-1380	225	"
1,2-Dipalmitoyl-sn-glycero -3-phosphocholine	- 120 ^c	218	ethanol

^aobtained from the sn-3 isomer^bthe n- π^* transition of the ester chromophore^cOther phosphatides also show a negative sign (see Table 3, page 21).

6. MASS SPECTROMETRIC ANALYSIS

6.1 Mass spectra of some glyceryl ethers.

A variety of natural and synthetic glyceryl ethers have been examined by MS^{21,22,23}. Most work concerning MS of glyceryl ethers containing a free hydroxyl group is to convert the hydroxyl group into a TMS-oxy group²³.

To confirm the proposed structure of the compounds synthesized for our study in section 5.3, we have examined some mass spectra of 1,2- and 1,3-O-dialkyl-glycerols. This work is described in paper VI.

We found that it was not necessary to transform these compounds into TMS-oxy derivatives, since the molecular ion (M)⁺ for 1,2-O-dialkyl- and 1,3-O-dialkyl glycerols were accompanied by ions due to the loss of methanol and water respectively (Fig. 20).

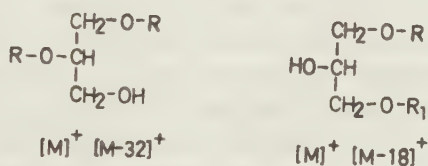


Fig. 20. The most major prominent ions in mass spectra for the identification of positions isomers of O-dialkyl glycerols.

This work shows that it is easy to distinguish positional isomers of aliphatic O-dialkyl glycerols, since their mass spectra differ.

6.2 *Mass spectrometric studies of isomeric hydroxy fatty acid pyrrolidides*

A wide variety of natural and synthetic long-chain fatty acids, usually in the form of the methyl ester derivatives, has been studied by mass spectrometry. Long-chain saturated esters are easily identified and are characterized by a prominent molecular ion and other significant ions.

When positional isomers are present, the EI (electron impact) fragmentation pattern in their mass spectra still gives valuable information, but in many cases they are indistinguishable from each other. This is true for positional isomers and homologues of unsaturated,²⁴ deuteriated²⁵ and to a certain extent hydroxy-substituted fatty acid methyl esters.^{26,27}

To improve the MS analysis of the latter fatty acids, if electron impact is chosen, the derivative could either be formed by A) modifying the hydroxy group or B) modifying the carboxylic acid group.

As examples of A) one can mention the work by Eglinton et al.,²⁸ who converted the hydroxy function to a TMS-oxy group, an acetoxy group or a trifluoroacetoxy group. Of these derivatives, the TMS group also gave the best results for polyhydroxy fatty acids. Even conversion of the hydroxy groups into methyl ethers should also be mentioned, as this derivative gives direct information about the site of the hydroxy groups. However, the polymethoxy acids yield mass spectra that are quite complicated due to competing fragmentation pathways. b) can be exemplified by the works of Andersson et al.²⁹ and Valicenti et al.,³⁰ who transformed the carboxylic acid group into a pyrrolidide, furthermore this approach has been thoroughly discussed and recommended as an alternative route method by Minniken.³¹

In the work, which is described in Paper VII, the mass spectra of all positional isomers of dl-hydroxyoctadecanoyl pyrrolidine are recorded, they are shown in Figures 21-25 (designated R 02 - R 18). Their different fragmentation pathways are discussed in the Paper.

Introduction of oxygen in the fatty acids chain of a pyrrolidide seems to have greater influence on the fragmentation pattern than other fatty acids pyrrolidide derivatives. That is since oxygen can easily donate an electron under EI due to the presence of an unshared electron pair, and competing fragmentations will obscure the typical pyrrolidide mass spectra. Long-chain hydroxy acids show a strong tendency toward cleavage of the bond at the carbon atom carrying the hydroxyl group. The most characteristic peak for identification results from the cleavage that occur on the side away from the pyrrolidide ring. This is exemplified by m/e 212 in the mass spectrum of dl-8-hydroxyoctadecanoyl pyrrolidide in Fig. 21.

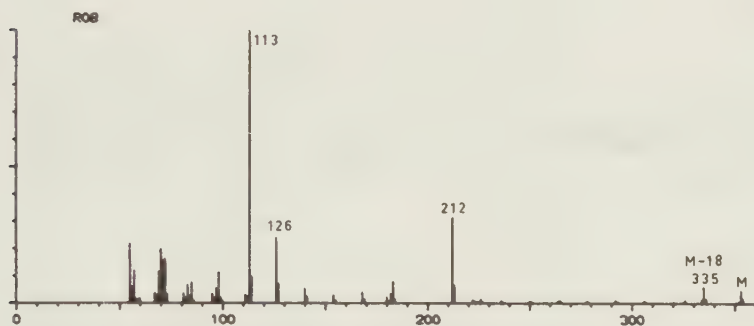


Fig. 21. The mass spectrum of dl-8-hydroxyoctadecanoyl pyrrolidene R 08

This work has shown that it is extremely easy to find the position of a monohydroxy group in a pyrrolidide fatty acid chain by EI mass spectrometry. All spectra of the investigated isomers differ and can be distinguished from each other.

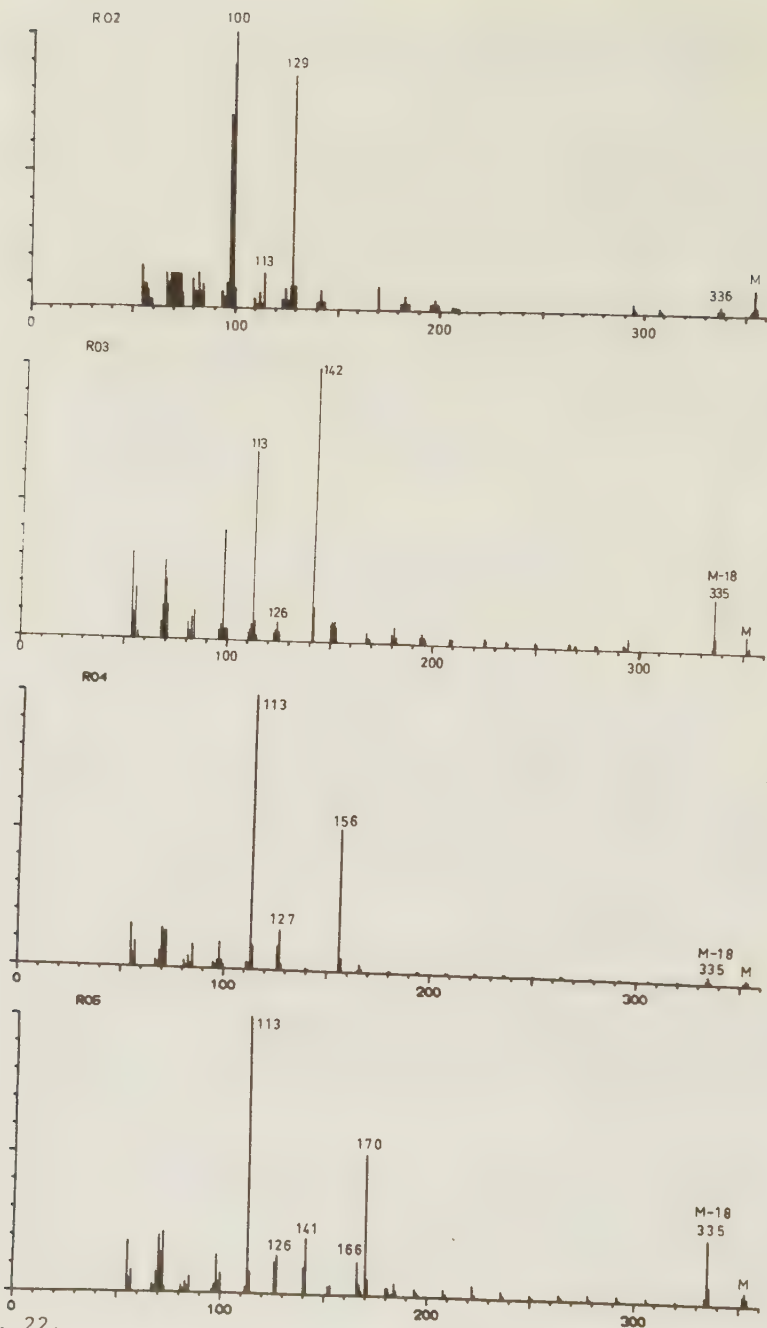


Fig. 22.

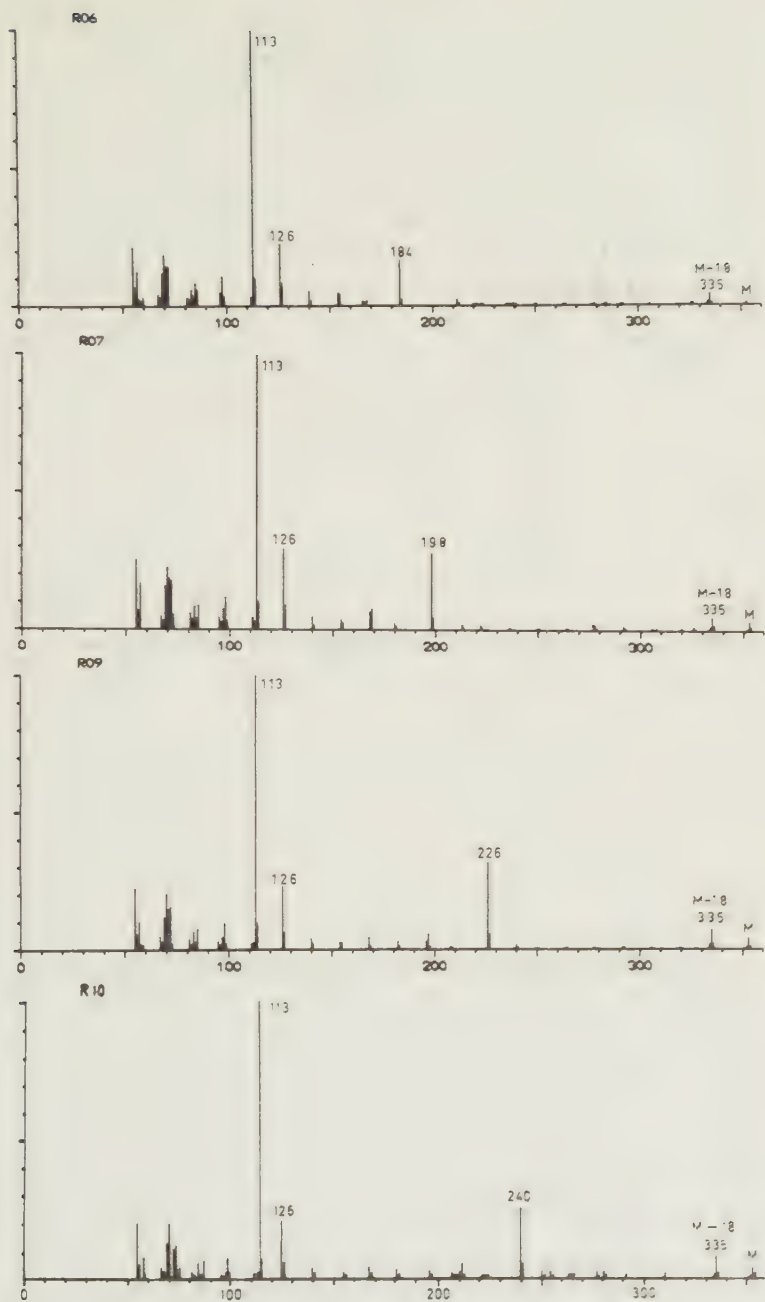


Fig. 23.

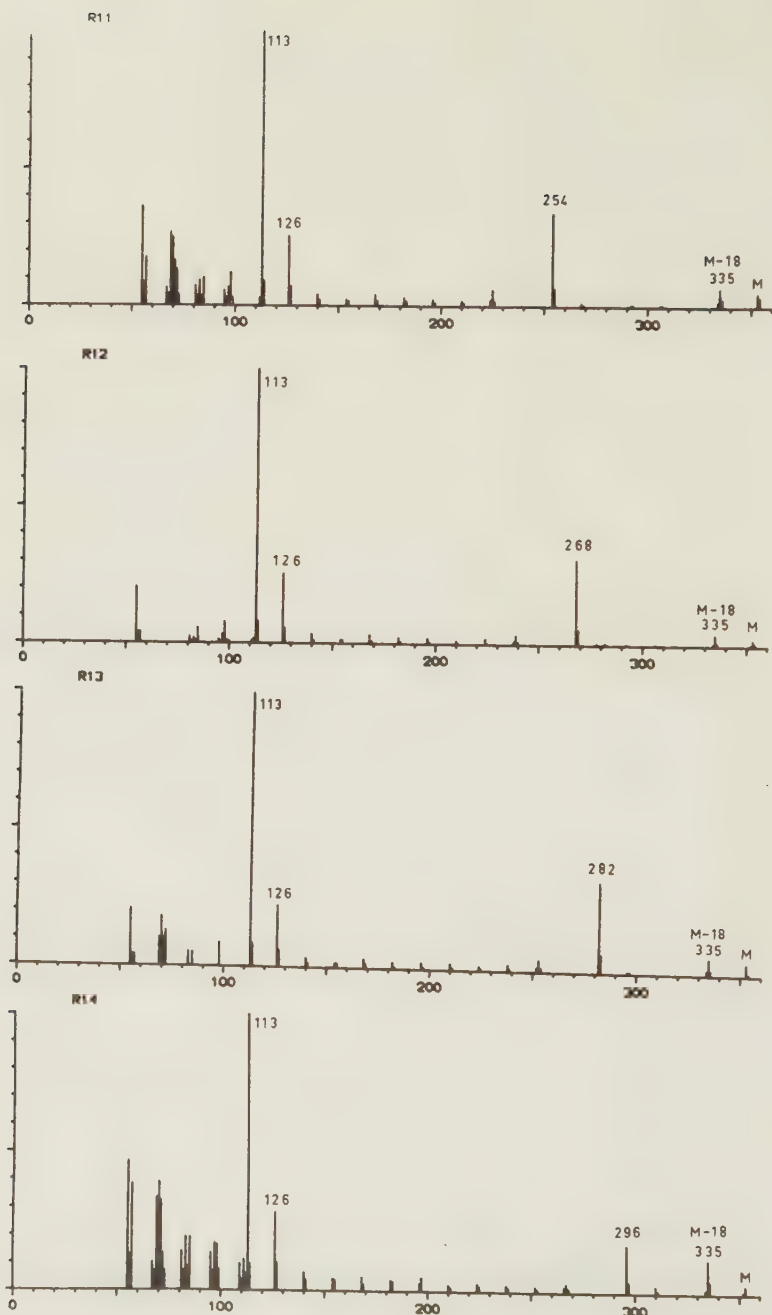


Fig. 24.

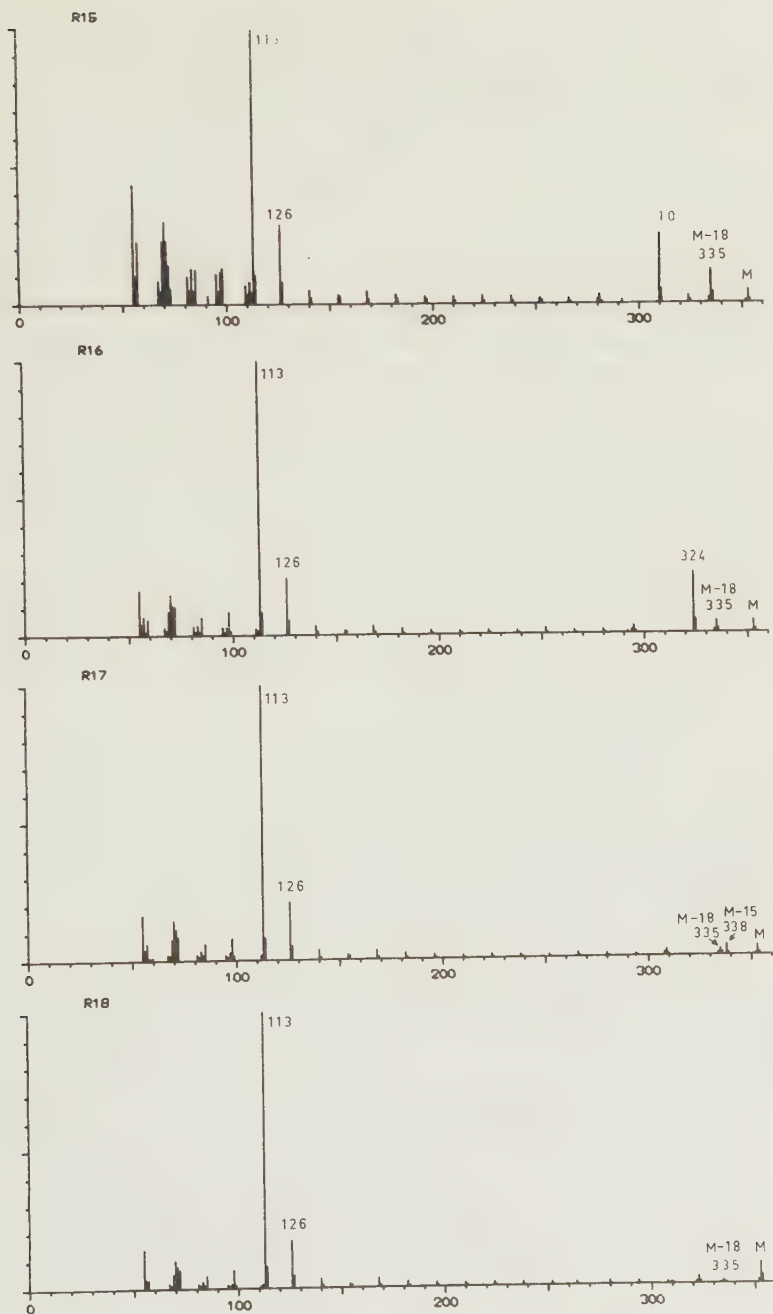


Fig. 25.

7. METHODS FOR STUDIES ON THE METABOLISM OF ENANTIOMERIC GLYCERIDES

7.1 *Introduction*

The stereospecific distribution of the fatty acids in natural and synthetic triacylglycerols can be determined by the method proposed by Brockerhoff.³² This analysis utilizes the positional and enantiomeric specificity of phospholipase A₂.

In an alternate procedure, Lands et al.³³ utilized diacylglycerol kinase from *Escherichia coli*, which selectively catalyzes the phosphorylation of the 1,2-diacyl-sn-glycerol, but not of the 2,3-diacyl-sn-glycerol. Also, diacylglycerol kinase from rat liver is stereospecific.³⁴ 1,2-Diacyl-sn-glycerol is also preferentially utilized in phosphatidylcholine synthesis via choline phosphotransferase, since the transfer of phosphorylcholine from cytidine diphosphocholine was stimulated by the addition of 1,2-diacyl-sn-glycerol, but not with the sn-2,3-isomer.³⁵

To elucidate the mechanism of lipase action, several synthetic compounds have been used such as radioactive and stable isotope-labelled molecules and among triacylglycerol analogues, compounds containing ether linkages,^{36,37} different polyhydric alcohols^{38,39} or amino groups.⁴⁰ Recently, acyl glycerols containing the thio ester bond have been suggested as suitable substrates for assay of lipase activity.

The degradation of enantiomeric triacyl glycerols and alkyldiacyl glycerols by lipases has been studied by several research groups.^{41,42,43,44} A preferential hydrolysis of fatty acids at position 1 has been demonstrated for lipoprotein lipases, whereas Akesson et al.,⁴⁴ using synthetic enantiomers of 1- and 3-O-alkyldiacyl-sn-glycerol, showed a similar positional specificity for the heparin-releasable hepatic lipase. Other types of stereospecificities have been demonstrated for microbial lipases (Akesson et al., unpublished).

7.2 *Intestinal absorption of lipids*

It has been demonstrated^{45,46} that during the degradation of triacyl glycerol in the intestine, mainly 2-monoacyl glycerol is formed. Skipski et al.⁴⁷ were the first to suggest that 2-monoacyl glycerol might be potential substrates in the resynthesis of triacyl glycerols. Studies on the structure of the resynthesized triacyl glycerols⁴⁸ indicated that 2-monoacyl glycerols might be absorbed intact since almost all fatty acids in position 2 of the ingested glycerides were not exchanged during the absorption. The possible differences in the absorption of fatty acids at positions 1 and 3 have not been studied to any great extent. In a study where enantiomeric 1-O-alkyl-2,3-diacyl glycerols were fed to rats, no such difference was found, although the isolated chyle lipids were not analysed by spectroscopic techniques.⁴⁵ Similarly, no optically active triacyl glycerols could be detected in chyle lipids after feeding with 1,2-dilauroyl-3-oleoyl-sn-glycerol.⁴⁵

7.3 *Triacyl glycerol analogue with thioester bond for the study on stereospecificity in lipid absorption*

Since triacyl-3-thio-sn-glycerols have considerably higher rotations than triacyl glycerols and alkyl diacyl glycerols (Table 1, page 12), we have used such a compound for further studies on the stereospecificities in lipid absorption (Paper VIII).

A triacyl glycerol analogue, rac-1,2-dioleoyl-3-S-oleoyl-3-thio-glycerol, was fed to rats and chyle acyl glycerols were analyzed. Triacyl glycerol was the dominant chyle lipid, but X-triacyl-1-thio glycerol constituted approximately 6 % of the total chyle lipids.

To determine the stereochemistry of this compound, ORD and CD spectra were studied. The shape of the ORD spectra (Fig. 26) for chyle X-triacyl-1-thioglycerol was the same as that obtained for trioleoyl-3-thio-sn-glycerol except that

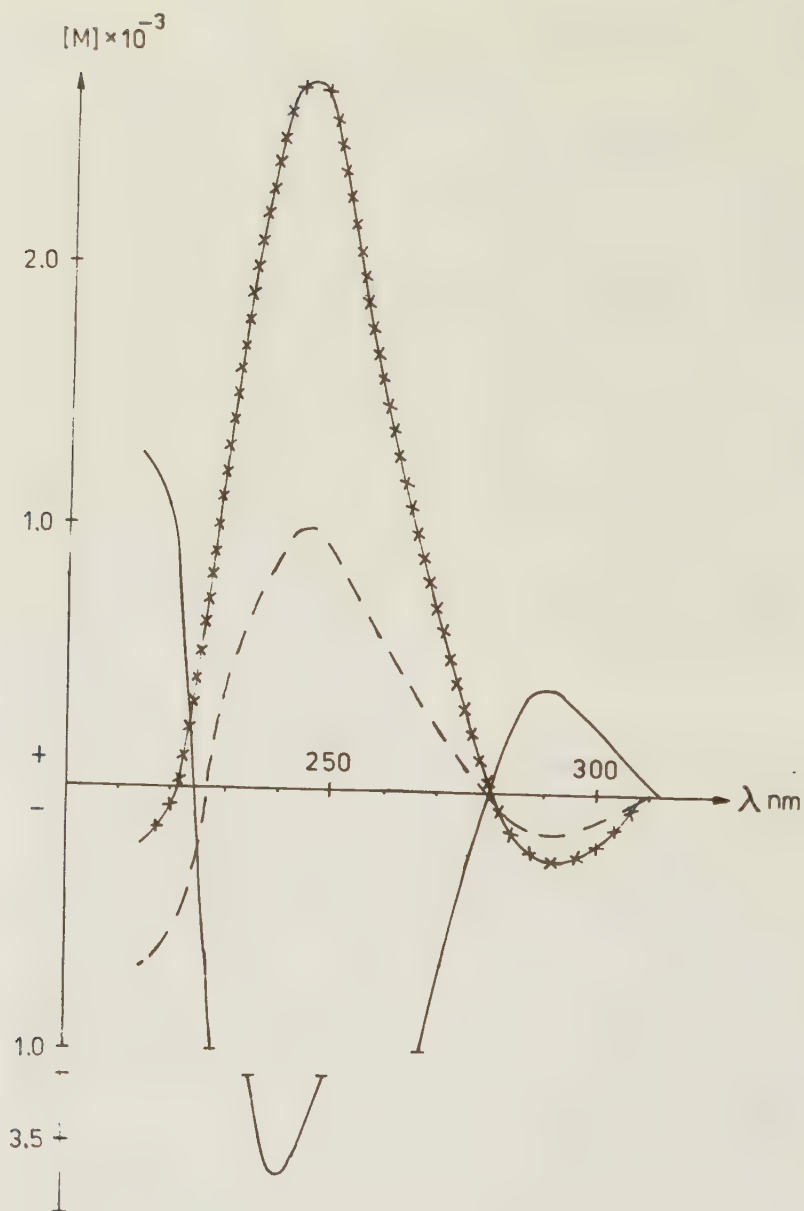


Fig. 26. ORD spectra (hexane) of trioleoyl-3-thio-sn-glycerol (—) and chyleX-triacyl-1-thioglycerol from expt. I (-----) and expt. II (+++++)

they were inverted. The rotations were however lower than that for the synthetic compound, indicating that the isolated samples did not contain only the 1-thio-sn-glycerol isomer, but instead a mixture of this isomer and the 3-thio-sn-glycerol isomer. The proportions of triacyl-1-thio-sn-glycerol/triacyl-3-thio-sn-glycerol isomers were 63/37 and 78/27 in two experiments, as determined by CD.

As discussed elsewhere,⁴⁹ the stereospecificity may reflect a preferential utilization of 2-acyl-1-thio-sn-glycerol rather than 2-acyl-3-thio-sn-glycerol in intestinal lipid biosynthesis but alternate mechanisms are possible.

7.4 1,2-O-Dioleoyl-3-S-tetradecyl-3-thio-sn-glycerol-S-oxide as a triacyl glycerol analogue for the study on stereospecificity in lipid absorption

Diacyl-3-O-alkyl glycerols, as mentioned above, have been used for the assay of lipase activity. These compounds have, however, too low optical activity to be useful for studies on small amounts of biological samples by ORD and CD.

For this purpose, we have used an analogue, rac-1,2-dioleoyl-3-S-tetradecyl-3-thioglycerol-S-oxide, which was prepared as described in Paper III. As described in Paper IX, this compound was fed to rats, and chyle lipids were analyzed. Triacyl glycerol was the major component but X-1,2-O-diacyl-3-S-tetradecyl-S-oxide constituted 8 % of the lipid weight. It was resolved by TLC into its two diastereoisomers, and each one was analyzed for the proportions of 1-thio isomer/3-thio isomer by ORD and CD, as described above.

The 1-thio isomer dominated for both diastereo isomers. This stereospecificity is most probably due to an acyl transferase, since 1-O-alkyl-sn-glycerol but not 3-O-alkyl-sn-glycerol is acylated in the intestinal mucosa.⁵⁰ In addition, the amount of diastereomer A (with S,S-configuration) was twice that of distereomer B (with S,R-configuration) in all experiments. Whether this selectivity is also exerted at the acyl transferase level is unknown.

Some evidence suggests that these compounds are synthesized analogous to diacyl-alkyl glycerol,⁵¹ and therefore a 1-thio-sn-glycerol configuration would be expected.

The studies mentioned in this section show that the stereochemical configuration of lipids isolated from biological material can be assessed by ORD and CD, with only approximately 2 mg of substance.

7.5 Stereospecificity of phospholipases

Phospholipases are useful tools in the analysis of lipids and they also have applications in synthetic work.

The reactive sites of phospholipids in the hydrolysis of different enzymes are given in Fig. 27.

Phospholipase A₂ can split off the fatty acid at position 2 of the 3-sn-glycerophospholipid, but not of 1-sn-glycerophospholipid.⁵² For phospholipase D, the available information is more difficult to interpret. Davidson et al.⁵³ found that rac-1,2-dipalmitoyl-glycero-3-phosphocholine was completely hydrolyzed by phospholipase D, although at a slower rate than for 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, but de Haas et al.⁵² reported that 2,3-diacyl-sn-glycero-1-phosphocholine resisted hydrolysis.

Due to these discrepancies we reinvestigated the stereospecificity of phospholipase D using the CD technique.

Rac-1,2-dioleoyl-glycero-3-phosphocholine was hydrolyzed by phospholipase D (Åkesson et al., unpublished result), whereby the choline group is split off. The resulting X-1,2-dioleoyl-glycero-3-phosphate and X-1,2-dioleoyl-glycero-3-phosphocholine were analyzed for their stereoconfiguration by CD. The preliminary results showed that 1,2-dioleoyl-sn-glycero-3-phosphate and 2,3-dioleoyl-sn-glycero-1-phosphocholine were the dominant isomers.

The results indicate that phospholipase D preferentially hydrolyses the sn-3 isomer, but further experiments are necessary. This investigation will be continued.

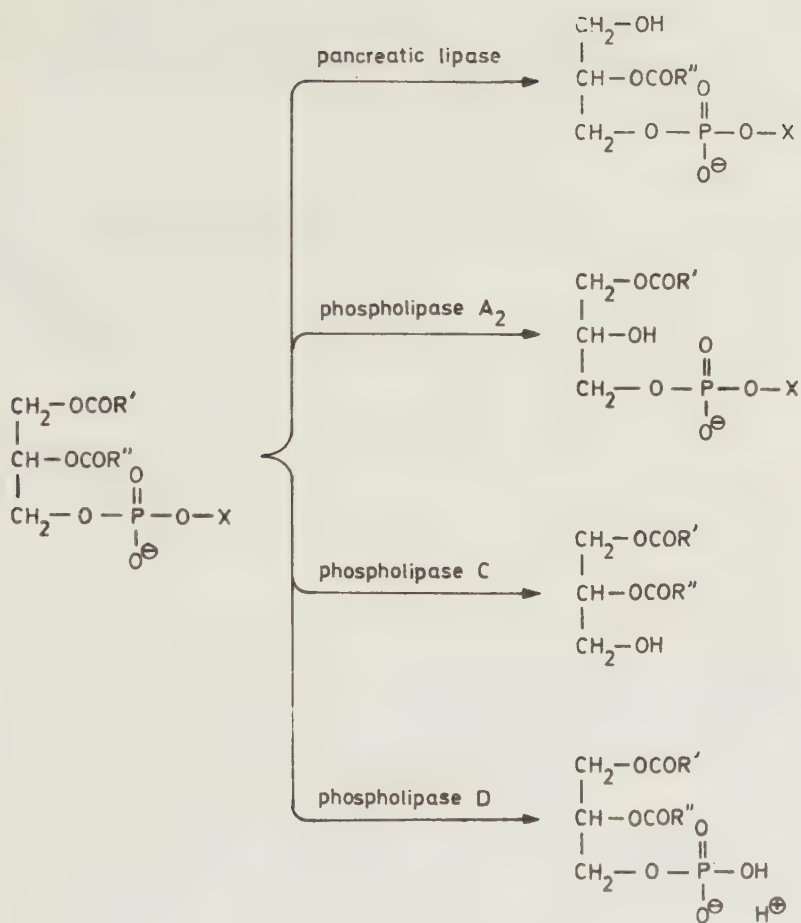


Fig. 27 . Enzymatic hydrolysis of glycerolipids
(X = choline etc.)

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